

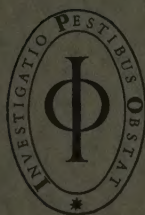
INSTITUUT VOOR PLANTENZIEKTENKUNDIG ONDERZOEK
WAGENINGEN, NEDERLAND
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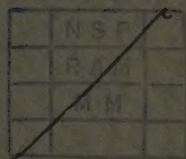
**HEAT TREATMENT AND SUBSTANCES INHIBITING
VIRUS MULTIPLICATION, IN MERISTEM CULTURE
TO OBTAIN VIRUS-FREE PLANTS**

DOOR

F. QUAK



Done from original



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Heat Treatment and Substances Inhibiting Virus Multiplication, in Meristem Culture to Obtain Virus-free Plants.

by
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Abstract—By means of the method of meristem culture, as developed by MOREL and MARTIN (1955), it is possible to grow virus-free plants from virus-infected ones. In certain cases, however, the virus-free part of the apical meristem proves to be so small that in order to get a satisfactorily growing meristem, it is necessary to isolate a bigger piece of tissue than the part that is virus-free. Thus the meristem culture will not result in a virus-free plant. In these cases application of substances that inhibit virus multiplication can be valuable. Substances, such as thiouracil, 2,4-D and IAA, may either be applied to the plant of which afterwards the meristem will be isolated, or added to the nutrient medium of the developing meristem. Favourable results have been obtained with the *X*-infected varieties, 'Bintje', 'Kennebec' and 'Valenciana,' and with the variety Eersteling which is carrier of the viruses *X* and *S*.

As heat treatment of virus-infected carnations does not always free the plants from virus, a modified meristem culture was applied on heat-treated carnations. The procedure resulted in virus-free plants.

Application de substances inhibant la multiplication de virus et utilisation de la chaleur, pour la guérison de plantes atteintes de maladie à virus par la méthode de culture de méristème.

Sommaire—En appliquant la méthode de culture de méristème, développée par MOREL et MARTIN, on peut obtenir des plantes saines à partir de plantes atteintes d'une maladie à virus. Ainsi on effectue une propagation par voie végétative sans que le virus se répande. Dans certains cas la partie du méristème apical qui est sans virus est tellement petite que, pour en obtenir une culture, il faut isoler une partie plus grande que celle qui est indemne de virus. Ainsi la culture de méristème ne produira pas de plante saine.

Dans ce cas l'application de substances qui inhibent la multiplication du virus peut être intéressante. Le thiouracil, le 2,4-D et l'AIA peuvent être appliquées aux plantes dont le méristème sera isolé ultérieurement, ou bien ajoutées au milieu nutritif du méristème. Des résultats favorables ont été obtenus avec les variétés de pomme de terre 'Bintje,' 'Kennebec' et 'Valenciana', infectées de virus *X* et avec la variété 'Eersteling', qui est porteuse des virus *X* et *S*.

D'autre part, une méthode modifiée de culture de méristème a été appliquée à des oeillets traités par la chaleur. Le procédé a permis d'obtenir des plantes saines.

Anwendung von Wärmebehandlung und virusvermehrungshemmenden Stoffen auf Meristemkultur zur Züchtung virusfreier Pflanzen.

Zusammenfassung—Mit Hilfe der Methode der Meristemkultur, welche von MOREL und MARTIN entwickelt worden ist, ist es möglich, virusfreie Pflanzen aus viruskranken Pflanzen zu gewinnen; es erfolgt eine vegetative Vermehrung ohne Virusübertragung. Es gibt jedoch Fälle, wo der virusfreie Teil des Meristems zu klein ist, um eine wachsende Kultur zu geben, sodass ein grösseres Stück als das virusfreie isoliert werden muss. Solch eine Meristemkultur wird keine virusfreie Pflanzen liefern.

In diesen Fällen kann Anwendung von Stoffen mit virusvermehrungshemmender Wirkung Wert haben. Stoffe wie Thiouracil, 2,4-D und Indolylessigsäure kann man entweder auf die Pflanzen anwenden, deren Meristem später zu isolieren ist, oder zum Nährboden des Meristems hinzufügen. Gute Resultate wurden mit den Kartoffelsorten ' Bintje,' ' Kennebec' und ' Valencianau' und mit der Sorte 'Eersteling', erzielt, die Träger der Viren X und S ist.

Da die Wärmebehandlung von viruskranken Nelken diese Pflanzen nicht immer virusfrei macht, wurde eine modifizierte Meristemkultur an wärmebehandelten Nelken durchgeführt. In dieser Weise entstanden virusfreie Pflanzen.

MANY valuable varieties of horticultural plants have been lost because virus diseases have spread throughout the clones and because for a long time no means was known of freeing fully infected vegetative clones from viruses. Other varieties, however, have been so valuable horticulturally that they have continued to be grown despite the fact that they were known to be fully virus-infected. In recent years it has been found possible to free some plants from virus infection by growing them at high temperatures, but there are still many plants which have not responded to this method. In the present paper some further methods or combinations of methods are described by means of which plants have been freed from virus infection.

Most, though by no means all, viruses occur in all the different types of tissue of the host plant. However, marked differences are often found in the concentration of virus in the various parts of the plants. This has been reported by, among others, LIMASSET, *et al.*⁽¹⁾ and LIMASSET and CORNUET⁽²⁾ who used tobacco plants infected with TMV. The assays they made of the virus concentration in various parts of the infected plants showed that this concentration is low in the younger leaves and increases as the leaves mature, to decrease again in the old leaves. They also tested excised apical meristems of infected tobacco plants, by inoculation on *Nicotiana glutinosa*, and found that these often did not produce infections on this test plant. They therefore assumed that the apical meristems were virus free. Results obtained later by other workers support this assumption.

However, other experiments have been reported which seem to contradict the results of LIMASSET, *et al.*⁽¹⁾ For example, SHEFFIELD⁽³⁾ excised apical meristems of tobacco and tomato plants infected with TMV and demonstrated the presence of the virus in these meristems. It is in fact very difficult to excise the apical meristem without contaminating it with virus, especially when dealing with TMV which occurs so abundantly in infected cells. On the other hand, negative results from inoculating isolated meristems from *Nicotiana glutinosa* may not prove the absence of the virus. Some virus particles may be present and

yet fail to cause local lesions on the test plant. Nevertheless there is enough evidence now to indicate that the apical meristems of infected plants may in a number of cases be virus free. Although the general validity of this has up till now not been proved, it is on this assumption that meristem culture is based. It provides us with a means of vegetative propagation which gives the opportunity of growing healthy plants from virus-infected ones. The method was first used by MOREL and MARTIN⁽⁴⁾ on varieties of dahlia, the culture of which was no longer profitable because of virus infection. They isolated the meristems of shoots of dahlia tubers, previously disinfected in a solution of calcium hypochlorite. These meristems were placed on the surface of a nutrient agar in small Pyrex tubes. On this sterile medium some of the meristems developed into small plantlets. These finally grew into normal-sized plants that could be tested for virus infection. A number of them proved to be virus free and were used to produce healthy stocks. The method of meristem culture has also been found to give favourable results, when applied to other species, as was our experience with the potato viruses *A* and *Y* and with leaf roll virus. The meristems were excised from sprouts on potato tubers and were 100–150 μ in diameter. In our experiments as a rule about 10 per cent of the isolations grew. Growth did not always start immediately. Meristems excised in spring usually grew rather quickly, whereas those isolated in autumn or in winter often developed only after a dormancy period of several months. When the plantlets were about 3 cm in length they were transferred into a light soil mixture and kept under glass for several weeks. During this time the almost leafless little stems developed the first leaves and some months later the potato plants formed were big enough to be tested for virus.

We found that with some viruses the virus-free part of the meristem was extremely small. To obtain a satisfactorily growing meristem it was often necessary to excise a piece of tissue larger than the virus-free part and in such cases, of course, meristem culture could not result in a virus-free plant. Potato virus *X* proved to be such a virus, occurring higher up in the tip of the host plant than, for example, the viruses *A* and *Y* and leaf roll virus. This, together with the fact that some potato varieties do not grow well on media that are quite satisfactory for others, made it difficult and sometimes impossible to get virus-free plants from meristems. In these cases applications of chemicals having an inhibiting effect on virus multiplication were found to be of value.

In further work with potato varieties infected with virus *X*, we used a nutrient agar made according to the formula of WHITE,⁽⁵⁾ to which we added either 10 p.p.m. thiouracil, 0.1 p.p.m. 2, 4-D or 0.1 p.p.m. IAA. Thiouracil inhibits virus multiplication, 2, 4-D and IAA are growth-promoting substances that in certain cases are found to have an inhibiting effect on virus multiplication. Meristems were grown on those media until they had produced plantlets of 1–2 cm length. From these plantlets tips of 1–2 mm were taken and placed in a new tube partially filled with the same nutrient. This procedure was repeated several times. The idea was that as a consequence of the virus multiplication being inhibited by the chemical, the virus-free part of the meristem would increase, so that finally a virus-free tip could be isolated. However, on these media the growth of the plantlets was rather poor, especially on those containing thiouracil. Only a few of them produced plants of normal size. For this reason the inhibitors were also applied in other ways.

Young potato plants were sprayed daily during four weeks with a solution of 100 p.p.m. thiouracil in water, after which treatment their tips were isolated. These tips were somewhat bigger than the usually isolated meristems and also included some leaf primordia. This meant that they developed more quickly and reached the stage sooner at which they could be tested for the presence of virus.

A third way of applying virus inhibitors was tried as follows: sprouts were taken from germinating potato tubers and were grown for 6 weeks on sand that was watered with solutions of inhibitors. After this period tips were isolated which again consisted of the meristem and some leaf primordia.

The three methods mentioned were applied to *X*-infected plants of the varieties 'Bintje', 'Kennebec' and 'Valenciana' and resulted in virus-free plants of these varieties. We also used these methods on the variety 'Eersteling'. This variety is a symptomless carrier of virus *X* and virus *S*. It had always resisted our numerous efforts to free it from virus by means of meristem culture. Before concluding that here we had a meristem that was not virus-free, we decided to try inhibitors. Of the three chemicals used only 2, 4-D had a favourable effect. A number of plantlets, grown on the 2, 4-D containing medium, eventually produced six potato plants in which no virus could be demonstrated, either serologically or by means of the indicator plant *Gomphrena globosa*. Two months later the testing was repeated. In four of the plants virus *X* was then found to be present, but two plants reacted negatively in several tests.

From this result and similar ones with the varieties Bintje, Kennebec and Valenciana, the conclusion was drawn that application of certain chemicals could give a valuable extension to meristem culture as a means of obtaining virus-free plants.

In one instance carnation meristem culture was combined successfully with heat treatment. Two varieties important in the carnation-growing district of the Netherlands, namely Pink Sim and Harvest Moon, were known to be completely infected with viruses causing a mottle and mosaic. In severely infected plants flower production is reduced and the value of cuttings decreases considerably when they show symptoms. Although damage may be restricted by careful selection, SCHOLTEN and BELGRAVER⁽⁶⁾ tried to obtain healthy material by means of heat treatment. The result was not satisfactory: when shoots, grown at a temperature of 40 °C, were tested serologically, it was found that, although the upper parts of the cuttings were virus free, the lower ends often contained virus. Moreover, the negatively-reacting cuttings often developed into plants the sap of which later gave positive serological reactions. We decided to apply modified meristem culture on plants that had been kept for 6-8 weeks at a temperature of 40 °C. As former results indicated that heat treatment "kept" the virus away from the tips of the plants, somewhat larger tips than usual were isolated, including not only the meristem and leaf primordia, but also two small leaves, the whole being about 1 mm in length. The growth of these tips was, as was to be expected, considerably better than that of the meristems. Root formation was stimulated by the addition of 1 p.p.m. α -NAA to the nutrient. After some months carnation plants of normal size were obtained. Sap from leaves taken at different heights on the plants was tested serologically. No reaction, indicating the presence of virus, was obtained. The sap was also inoculated into *Dianthus barbatus*. This plant reacts to the carnation viruses ringspot,

mottle and vein mottle, but not to the latent carnation virus.⁽⁷⁾ The inoculated plants showed no reactions. Eight months later the testing was repeated and the plants proved again to be free from ringspot, mottle and vein mottle viruses. Although it is not known to what extent the latent carnation virus occurs in the Netherlands we tested the plants for the presence of this virus by means of potato virus *S* antiserum.⁽⁷⁾ We did not obtain any evidence of its presence in our carnation plants. The plants are now being propagated in order to provide a stock of virus-free plants from which carnation growers may obtain virus-free cuttings.

Although our knowledge of the different carnation viruses is not yet complete, the present results permit the conclusion that from virus-infected plants, subjected to heat treatment, virus-free plants can be obtained by means of "tip isolation".

Meristem culture is laborious and time consuming which diminishes its value for practical purposes, however interesting its theoretical aspects may be. Combining it with heat treatment and the use of inhibitors of virus multiplication may help to make it more applicable in the practice of plant propagation.

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